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# Photopharmacology: beyond proof of principle

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## ABSTRACT:

Pharmacotherapy is often severely hindered by issues related to poor drug selectivity, including side-effects, environmental toxicity and the emergence of resistance. Lack of selectivity is caused by the inability to control drug activity in time and space. Photopharmacology aims at solving these issues by incorporating photoswitchable groups into the molecular structure of bioactive compounds. These switching units allow for the use of light as an external control element for pharmacological activity, which can be delivered with very high spatiotemporal precision. This perspective presents the reader with the current state and outlook on photopharmacology. In particular, the principles behind photoregulation of bioactivity, the challenges of molecular design and the possible therapeutic scenarios are discussed.

## 1. Introduction

Pharmacotherapy offers the possibility to cure diseases and alleviate symptoms through the administration of drugs.<sup>1,2</sup> It improves quality of life and has contributed to what our world is today. However, there are many drug-related problems that are of a major concern to society. Poor drug selectivity can result in toxicity-related side effects,<sup>3</sup> but less obvious complications are frequent also, such as drug resistance (Figure 1).<sup>4,5</sup> These effects decrease the potential and limit the application of pharmacotherapy. Increasing the impact of pharmacotherapy requires exploration and establishment of novel molecular paradigms towards increasing the selectivity of drugs.<sup>6</sup>

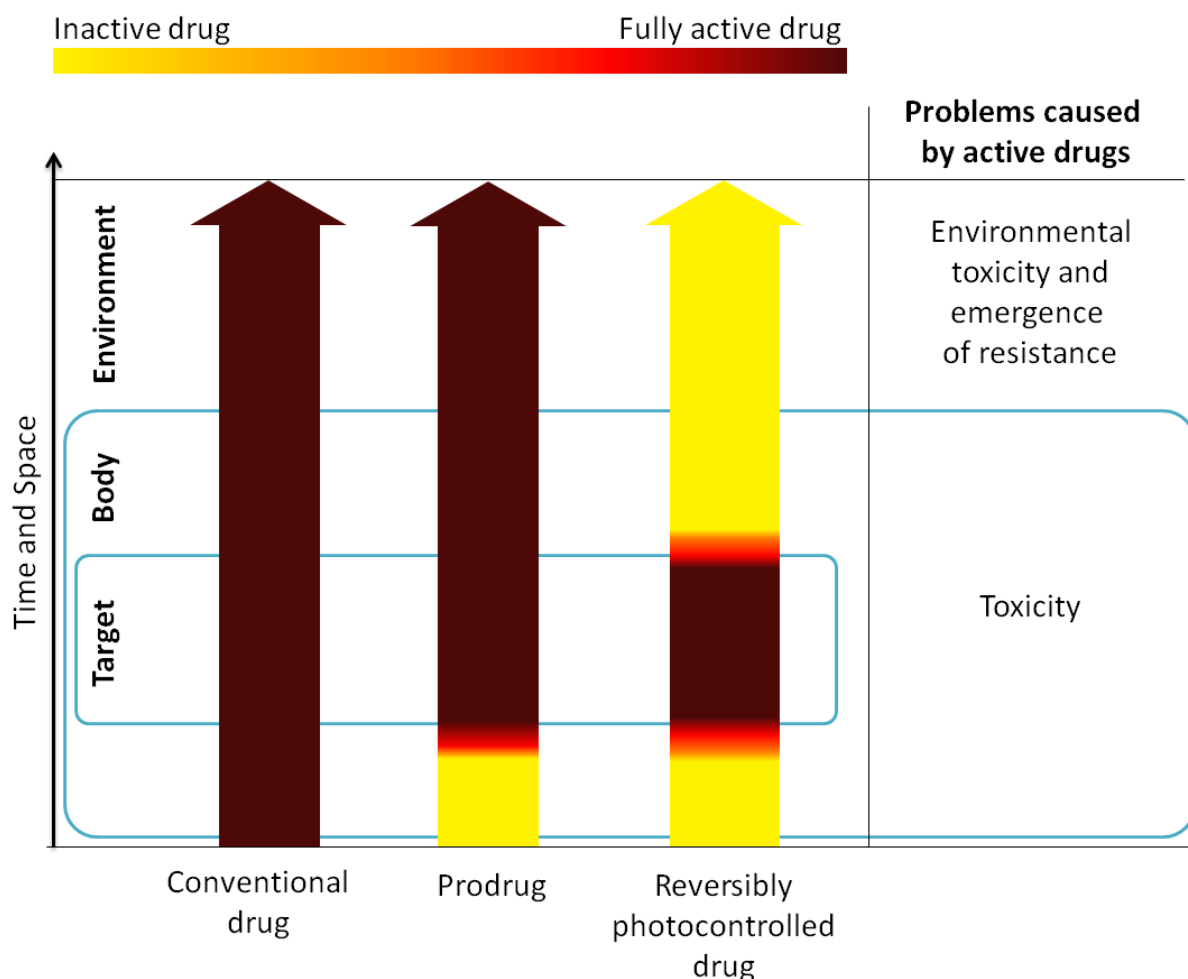
Poor drug selectivity stems from a drug's affinity for targets other than that intended.<sup>3</sup> This limitation is perhaps unsurprising when one considers that drugs interact with processes embedded in complex signaling and metabolic pathways.<sup>6,7</sup> Cytotoxic anti-neoplastic agents, such as vinca alkaloids and taxanes, while widely used in the treatment of various types of cancers, are infamous for their severe side effects.<sup>8</sup> The mechanism of action by which these anti-cancer drugs achieve their effect is usually by impairing mitosis,<sup>8</sup> which also affects healthy, fast-dividing cells causing adverse events, most notably, hair loss,<sup>9</sup> mucositis<sup>10</sup> and anemia.<sup>11</sup> To overcome these side effects, substantial efforts have been devoted to the development of targeted therapy,<sup>12,13</sup> but selectivity remains a major hurdle.<sup>14,15</sup>

Furthermore, poor selectivity lowers the threshold level of toxicity and thereby narrows the therapeutic window, leading to a decrease in allowable dose. This enforces suboptimal dosage, i.e. not using the full potential of a drug.<sup>16</sup> Over 85% of small molecule drugs in clinical research are discarded,<sup>17</sup> partially because of their limited therapeutic window. This means that many interesting drug-candidates, which potentially could improve present pharmacotherapy or even treat currently untreatable conditions, are discarded solely due to insufficient selectivity. Recent advances in drug discovery show that many effective pharmaceuticals act by modulation of multiple targets (network pharmacology).<sup>6</sup> This means a drug needs to interact with multiple sites simultaneously to gain maximum activity, but should still not interact with active sites in a manner that leads to side-effects. This polypharmacology complicates the selectivity problem further and calls for dynamic control of drug activity.

Another major issue in modern pharmacotherapy is drug resistance.<sup>4,5</sup> In particular, the use of antibacterial agents is under pressure because of the emergence of many resistant bacterial strains.<sup>5,18</sup> Bacterial resistance stems mainly from the build-up of antibiotic agents in the environment, both in community and hospital settings. As a result, the survival of sensitive bacteria in the environment is inhibited, promoting the growth of resistant strains. This phenomenon can be regarded as poor selectivity as well, where the drug is not only selective for bacteria causing an infection in a patient, but also acts on bacteria outside the body.

The problems caused by poor selectivity originate in uncontrolled drug activity in time and space, i.e. the drug is active at times and sites in- or outside the organism, when and where drug activity is not beneficial (Figure 1). This drawback provides an impetus for the search

for novel molecular approaches for dynamically controlling drug activity, bypassing the issues surrounding poor drug selectivity.



**Figure 1.** Drug activity over time and space for a conventional drug, prodrug and a reversibly photocontrolled drug. For conventional drugs, activity is present throughout the body of a patient during the time the drug is present, which may cause adverse effects; after excretion, the build-up of active drug can have a negative impact on the environment and/or lead to the emergence of drug-resistant pathogens. Some of the adverse effects can be avoided by using prodrugs, which liberate the active substance at a later stage. Adverse effects and environmental build-up of drugs might be avoided by using drugs whose activity can be controlled reversibly in space and time.

Light offers unparalleled opportunities as a non-invasive regulatory element for biological applications.<sup>19-27</sup> Firstly, it shows a great degree of orthogonality towards most elements of chemical and biochemical systems. In contrast to chemicals, which are used for regulating biological processes, photons do not cause contamination of the studied object and have low or negligible toxicity. Secondly, light can be delivered with very high spatial and temporal precision, which is of paramount importance for controlling the action of bioactive compounds (Figure 1). Finally, light can be regulated in a qualitative and quantitative manner, by adjusting wavelength and intensity, respectively.

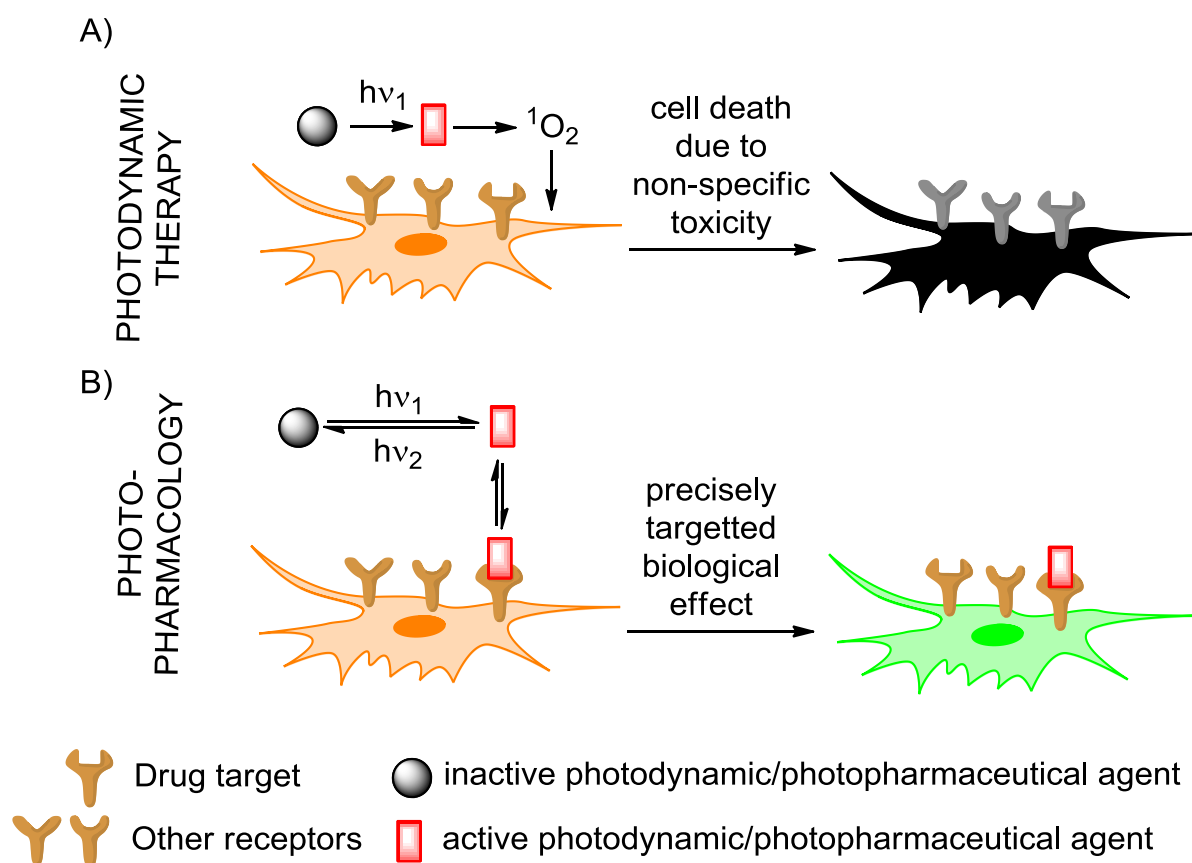
The aim of this perspective is to summarize the state of the art and provide an outlook on the molecular methods that allow for drug selectivity to be increased by controlling activity with light. The first part of this perspective focuses on the design principles while the second part serves to illustrate examples from the recent literature.

## **2. Using light for controlling biological processes: the concept of photopharmacology**

Remote control over the activity of pharmaceuticals using light has been achieved by modifying a functional group within the drug with a photolysable moiety.<sup>19,28,29</sup> Examples of this approach include a photoprotected prodrug of paclitaxel<sup>30</sup> and a light-activated steroid hormone.<sup>31</sup> However, a major limitation of this approach is that photodeprotection does not enable reversible control over the drug's activity. It only enables an increase or decrease in the activity of a drug a single time, but it is not possible to switch between a state of low activity and one of high activity reversibly.

Dynamic control of activity is highly desirable as it enables full control over the activity of a drug both temporarily and spatially. Dynamic optical control over biological functions can be achieved using optogenetics.<sup>32,33</sup> This “Nature method of the year 2010”<sup>34</sup> relies on the expression of photoresponsive proteins such as bacteriorhodopsin<sup>35</sup> and halorhodopsin.<sup>36</sup> Optogenetics has mainly, but not exclusively, proven useful in the field of neuroscience, with examples of optical control of brain function in intact brain tissue<sup>37</sup> and of the movement of animals.<sup>38</sup> It is without doubt that optogenetics has made a major contribution to advance our understanding of biological processes and has become an important research tool in such investigations. However, the necessity for genetic manipulation limits this method currently to a research setting.

Photodynamic therapy (PDT)<sup>39</sup> is a clinically applied protocol that relies on the formation of reactive oxygen species (ROS) by using photosensitizers that transfer energy from their excited triplet state to  $^3\text{O}_2$  to generate singlet oxygen ( $^1\text{O}_2$ ) in living tissue (Figure 2A). ROS, primarily  $^1\text{O}_2$ , cause direct and non-specific damage to the cells containing the photosensitizer when irradiated with visible light.<sup>40</sup> In this respect, PDT is not inherently selective for a chosen molecular target, which is connected to the disease. Also, its application is limited to situations where cell death must be evoked, such as the destruction of tumors or infected tissues.



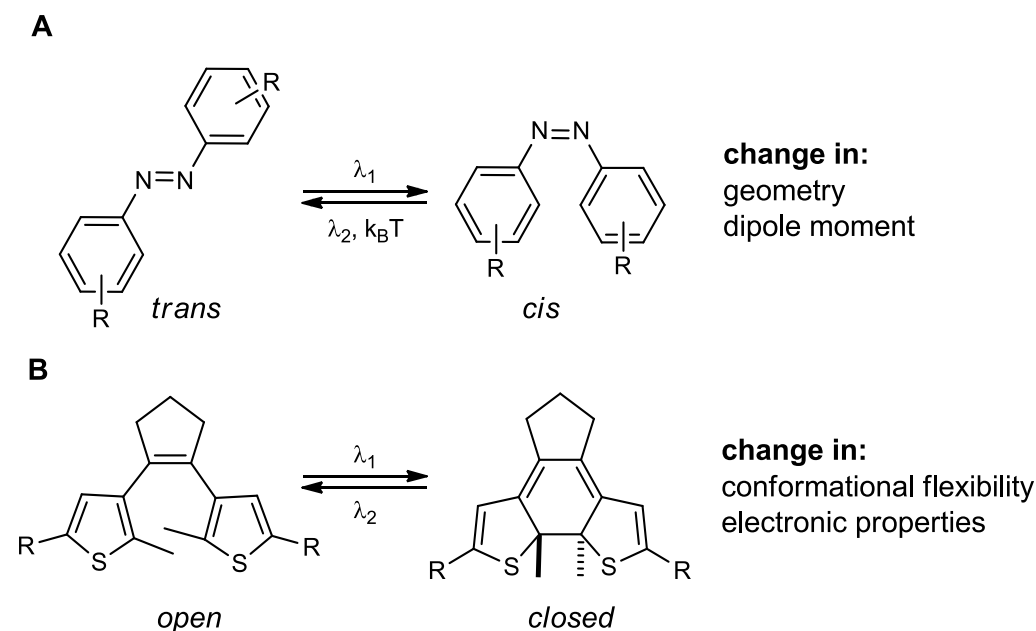
**Figure 2.** Basic principle of photodynamic therapy (PDT) and of photopharmacology. A) PDT agent can be excited to the triplet state locally with light. If the triplet state has sufficient energy, it will result in the production of singlet oxygen ( $^1\text{O}_2$ ), which leads to the death of the surrounding cells; B) Light-induced isomerization of a photopharmaceutical agent from an inactive to an active state results in abrupt, localized increase in the concentration of the active form of the drug, which interacts with its target selectively.

The principle of photopharmacology is the introduction of a photoswitchable unit into the molecular structure of the bioactive compound itself (Figure 2B). Dynamic control is achieved through addressing small photoresponsive molecule, and in a sense is comparable to classical pharmacotherapy, making this method potentially interesting for medical applications. Specificity to the selected target is intrinsic to this approach, as it employs a highly optimized design of a selective, bioactive compound, into which a responsive element is introduced.

Photopharmacological treatment is especially suitable in situations where the disease is localized. The action on exposed parts, such as skin or eyes, seems the most natural target. However, the advances in designing molecular photoswitches that respond to deeper-penetrating red light make it increasingly viable to treat other localized conditions such as infections and solid tumors.

Molecular photoswitches are compounds that, upon absorption of a photon, may undergo a reversible change in their structure and properties. While many classes of such light-responsive compounds have been developed,<sup>41</sup> two basic designs have attracted most

attention<sup>21</sup> in the field of biological applications: azobenzenes<sup>22</sup> (Figure 3A) and diarylethenes<sup>42</sup> (Figure 3B).



**Figure 3.** Photoisomerization of (A) azobenzenes and (B) diarylethenes.

The photoinduced *trans-cis* isomerization of azobenzenes is accompanied by a large change in geometry and a considerable change in polarity (the dipole moment changes by ~3 D). Relaxation to the thermodynamically more stable *trans*-isomer can be achieved by irradiation with light at a different wavelength or may proceed spontaneously, i.e. thermal isomerization. Diarylethenes contain a hexatriene moiety that can undergo reversible, photochemically induced cyclization, changing the flexibility of the molecule and its electronic properties (conjugation length). In general, both open and closed isomers are thermodynamically stable and can be interconverted using light of different wavelengths.

A drug's effectiveness depends on its pharmacokinetic and pharmacodynamic properties.<sup>43,44</sup> The pharmacokinetic properties determine how a drug is dispositioned around the body, which is determined by absorption, distribution, metabolism and excretion and can be estimated based on the polarity, lipophilicity and size of a pharmaceutically active compound, among other factors.<sup>43</sup> By incorporating a molecular photoswitch into the structure of a drug, dynamic control over these properties can be achieved using photoirradiation. For example, there is a distinct difference in the polarity and size of an azobenzene in its *trans* and *cis* states.<sup>45</sup> For a drug to reach its target, often distinct properties are required at various stages. For example, the polarity of a drug has a large influence on its pharmacokinetic properties and variation in polarity can be beneficial at various moments during drug distribution.<sup>46</sup> Dynamic control over these properties could potentially increase drug effectiveness.

Pharmacodynamic characteristics define how a drug interacts with its target.<sup>47</sup> Common drug targets are G Protein Coupled Receptors (GPCRs), enzymes and ion channels.<sup>7</sup> The molecular structure of a drug is often highly optimized to maximize drug affinity for these targets.<sup>7</sup>

When a photoswitchable moiety is introduced into the structure of a drug, its configuration can be changed upon switching. If the photoswitch is introduced in an appropriate manner, the drug can be switched between states of high and low affinity. In this way, a drug can be present in a state of low affinity when this is beneficial, reducing adverse effects. The drug can be switched to a state of high affinity when required, i.e. when a drug has reached its site of action (Figure 2B). This strategy can be employed to control the pharmacodynamic properties of a drug externally in order to increase selectivity.

### **3. Design principles for creating photocontrolled drugs**

In the following discussion on the design of photocontrolled drugs the focus is primarily on azobenzenes, since they are the most widely applied of photoresponsive elements in bioactive compounds. It is of note, however, that the discussion in regard to applications and design principles can be extended to other photochromic systems also.<sup>41</sup>

#### **3.1. Pharmacological considerations**

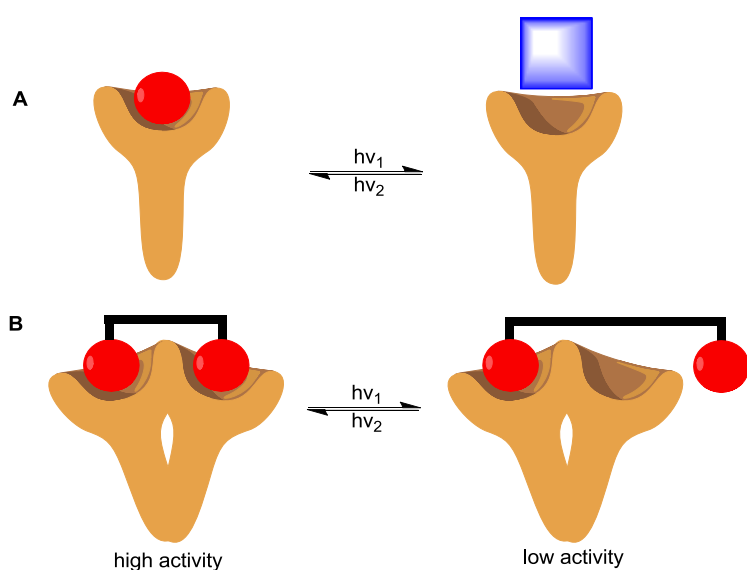
When incorporating a photoswitchable moiety within the structure of a pharmaceutically active compound, biological activity needs to be retained in at least one of the photoisomeric states. The structure of drugs is often highly optimized to attain maximum potency and efficacy.<sup>48</sup> Introducing a photoswitch into such an optimized structure will frequently lead to a decrease in activity. This might be a potential limitation when employing this method in the pharmaceutical industry, when moving beyond the proof of principle setting in academic laboratories. Therefore, further optimization of the structure is necessary once a photoswitch has been successfully introduced in order to regain high potency and efficacy. Furthermore, in order to minimize overall activity loss or maximize activity loss in only one of the photoisomeric states, it is sensible to study structure activity relationships (SARs) or perform crystal structure analysis of the drug-receptor complex to guide the design of a photoswitchable compound that shows a large difference between the two photoisomeric states. Finally, one has to consider the possible existence of and potential differences in the off-target effects of both photoisomeric forms of the drug.

Two strategies are generally taken when designing photopharmaceuticals. The first strategy is to couple a photoswitch to the pharmacophore (Figure 4A). The photoswitchable moiety can be partially incorporated into the pharmacophore, as was shown by the groups of Trauner<sup>49</sup> and Feringa<sup>50</sup> or appended to the pharmacophore, as reported by Fischer and coworkers.<sup>51</sup> These strategies rely on high affinity of the drug to the receptor in one of the photoisomeric states. Photoisomerization alters the shape and polarity of the drug, interfering with drug-receptor interactions, thereby resulting in a decrease in binding affinity. It may also be caused by a drug's inability to reach the receptor active site, because its shape, size or polarity after switching may not permit entrance to the binding pocket. In either case, the photopharmaceutical can be switched between a state of high and low activity.



A second strategy is applicable to multivalent drugs. These drugs consist of two or more pharmacophores, which are connected by a spacer unit.<sup>52</sup> The spacer provides the required rigidity and distance between the pharmacophores for optimal activity. Examples of multivalent drugs include bivalent opioid receptor agonists<sup>53</sup> and chromones.<sup>54</sup> A photoswitch can be introduced as the spacer, altering the rigidity of the spacer and the distance between pharmacophores upon photoisomerization, which leads to a change in drug activity (Figure 4B). This method has been exploited for the development of photoswitchable mast cell activation inhibitors<sup>55</sup> and photoswitchable ITAM peptidomimetics.<sup>56</sup> Since the optimal distance between the pharmacophores is of utmost importance to attain high activity, photoswitches that can realize a large change in this distance, and a concomitant change in activity, are necessary for this strategy to be feasible. Therefore, azobenzenes are the most widely used of switches for this purpose.

One potential problem, encountered when applying photopharmacology, is that the drug molecule might be prevented from switching when it is bound to the pharmacological target. Especially in cases where one isomer binds tightly to the receptor, the isomerization efficiency might not be large enough to realize photoisomerization. However, in most cases receptor binding is a dynamic event. At the moment the drug molecule leaves the binding pocket it will immediately be photoisomerized.



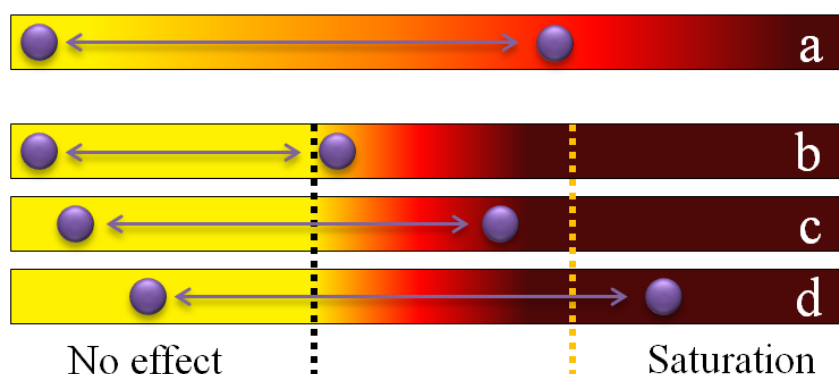
**Figure 4.** Schematic representation of the two major strategies employed for designing photopharmaceuticals. A) A photoswitch bound to a pharmacophore changes its properties upon switching and prevents binding in one of the states; B) A photoswitch is introduced between two ligands that interact with a multivalent target; isomerization of the photoswitch changes the distance between the ligands and prevents the multivalent interaction in one of the states.

### 3.2. Photoisomer ratio at photostationary state (PSS)

One of the crucial factors, that need to be considered when designing a responsive drug by incorporation of a photochromic unit, is the difference in relative concentration of the two isomers that can be addressed by light. For most of the used photoswitches, it is not possible to transform completely from the thermodynamically favored state to the unstable state upon irradiation.

In the case of azobenzenes, the *trans*-form is usually  $> 10$  kcal/mol<sup>57</sup> more stable than the *cis*-form, which means that in a thermally equilibrated sample only the former is present to any extent. Irradiation with light at a suitable wavelength causes a part of the population of molecules to switch to the *cis*-state. Recently a number of bidirectional azobenzenes has been reported in which this process is especially efficient and the irradiated state consists almost exclusively ( $>95\%$ ) of the thermodynamically less stable isomer.<sup>58-60</sup>

Bistable switching is of paramount importance for many applications of molecular photoswitches, in which the degree of isomerization translates directly to the biological effect. If one considers, for example, photocontrol of the catalytic activity of an enzyme by the covalent attachment of a molecular photoswitch,<sup>21,25</sup> it becomes apparent that, even if the isomerization of a single, enzyme-bound photochrome results in an on-off change in activity, the overall change in activity will be directly correlated to the percentage of the photochemically active compounds that undergo isomerization. This situation is represented in Figure 5a, in which the color intensity represents biological activity, and which is inspired by similar considerations with regard to photocaged compounds by Deiters.<sup>24</sup>



**Figure 5.** Photoswitching (purple arrow) between two states that differ in biological activity. The activity increases from yellow to dark red. a) When the molecular photoswitch is attached covalently to the target, there is a linear correlation between the percentage of photoswitches that undergo the isomerization and the change in catalytic activity; b-d) when the stoichiometry between the photoswitch and the target is not fixed, it is possible to achieve higher changes in activity by increasing the dose of a photoswitchable drug (from b to d). Even in the initial state (left sphere) some of the active form might be present, increasing its concentration, albeit below certain activity threshold (dashed black line), will not result in the biological response. Due to the steepness of the sigmoidal-shaped dose-response curve, it is in principle possible to find conditions (d) where the concentration of the active state (right sphere) is beyond the saturation point (dashed orange line) and complete on-off control can be achieved.

On the other hand, the use of photoswitches for controlling the bioactivity of drugs is, by definition, a non-covalent approach, i.e. it does not rely upon direct attachment of the photoactive compound to the drug's target. It means that the stoichiometry of the two is not fixed and by changing the concentration of the drug it is possible to optimize the response (Figure 5b-d) even if only part of the population of the photoswitchable drug undergoes isomerization. This effect is especially enhanced in the case of the steep, sigmoidal dose-response curves (Figure 5b-d), observed in pharmacology. These special features render photopharmacology an attractive approach among the numerous methods<sup>21</sup> that rely on using molecular photoswitches for the optical control of biological processes.

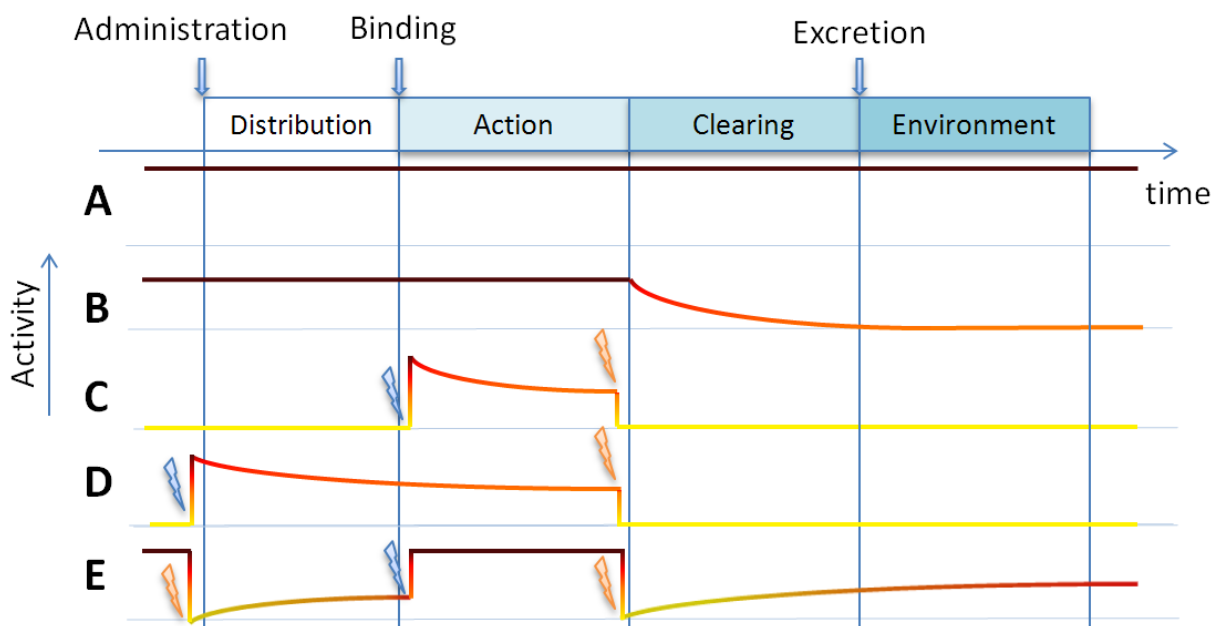
### 3.3. Wavelength tuning

The possibility of tuning the wavelength of light needed for photoisomerization of the photopharmacological agent is of crucial importance. Two factors determine the range of the spectrum that can be used: the damage caused by irradiation with UV light and the wavelength-dependence of the depth of tissue penetration.

The toxicity of UV light has been studied extensively and it has been shown that it is carcinogenic,<sup>61</sup> can cause mutations,<sup>62</sup> and induce cell apoptosis.<sup>63,64</sup> Therefore, UV light should be avoided in therapies and UV-switchable compounds cannot be used in the clinic for applications that involve the direct irradiation of a living tissue.

The depth of penetration of light into tissues is limited by both optical scattering and absorption by the endogenous chromophores.<sup>65</sup> In principle, the available wavelength range is between 600 and 1200 nm, with the absorption of hemoglobin preventing the use of shorter wavelengths and the absorption of water limiting the use of longer wavelengths.<sup>65</sup> While these values are strongly tissue-dependent, it is assumed that light at 630 nm can penetrate 1 cm of tissue, while for 800 nm the depth of penetration is approximately 2 cm.<sup>66</sup> While this is usually not enough for completely non-invasive irradiation, light can be delivered to most organs in the body using an optic fiber inserted through a small incision.<sup>67</sup>

An ideal situation would be offered if switching in both directions could be achieved with non-toxic and deep-penetrating light. This would enable therapeutic scenarios described in Figure 6, which require the use of two distinct wavelengths of light to 'turn on' and 'turn off' activity. Scenario A (1, Figure 6) represents the case of a normal pharmacological agent, which is excreted in an active, non-metabolized form. The case of a drug that undergoes partial metabolic degradation is represented by the second scenario (B, Figure 6). In both situations the drug is still present in its active form in spaces and times in which it is unnecessary, or may even be harmful.

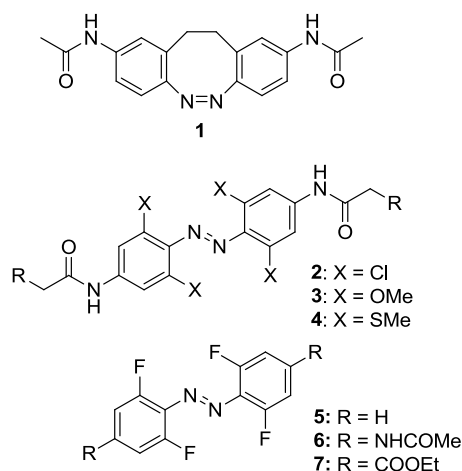


**Figure 6.** Possible therapeutic scenarios using photoswitchable bioactive compounds which can be switched between an on- and off-state with light of different wavelengths: A) Activity profile of a conventional drug that is not metabolized in the body; B) Activity profile of a conventional drug that is metabolized in the body; C) Photoactivation of a switchable drug on the site of action, followed by inactivation when it is being cleared from the body; D) Photoactivation of a switchable drug prior to administration, followed by inactivation when it is cleared from the body; E) Inactivation of a switchable drug prior to administration, subsequent activation at the site of action and final inactivation when it is being cleared from the body.

For example, cytotoxic compounds used in the chemotherapeutic treatment of cancers cause adverse effects,<sup>68</sup> which could be avoided by local activation of the drug within the tumor (Scenario C, Figure 6). A further problem is caused by the build-up of bioactive compounds in the environment, which has severe consequences in case of antibiotics<sup>5</sup> and hormones.<sup>69,70</sup> In such cases, using light to turn off the drug before it leaves the body (Scenario C, Figure 6) would prevent the excretion of active component. Alternatively, if the drug needs to be activated with potentially harmful UV light, this can be achieved safely by irradiation prior to administration (Scenario D, Figure 6). It has to be noted that upon excretion to the environment the compound would potentially be exposed to sunlight. This exposure is, however, not efficient enough for the buildup of significant content of the photoswitched form. Also, one has to consider that the access of sunlight to the spots in the environment such as soil or sewage systems is severely limited.

Both applications envisioned in scenarios C and D rely on the use of a compound, which exhibits activity in the thermodynamically unfavorable state. In the opposite case, where the isomer present after thermal equilibration is more active, one could envision using light of different wavelengths to sequentially turn the activity off before administration, turn it on

again at the place of action and again turn it off outside the place of action to avoid toxicity in other parts of the body (Scenario E, Figure 6).



**Figure 7.** Visible-light-switchable azobenzene-based photoswitches **1-7**.

In recent years, major advances have been made in understanding the design principles for the synthesis of azobenzenes that can be switched with visible light. Selected representative examples are shown in Figure 7. The optical properties of compounds **1-7** are summarized in Table 1. In every case, isomerization in both directions can be achieved by irradiation with visible light.

**Table 1.** Photochemical properties of azobenzene-based photoswitches **1-7**.

Compound	$\lambda_{\text{trans} \rightarrow \text{cis}}$	$\lambda_{\text{cis} \rightarrow \text{trans}}$	half life	reference
<b>1</b>	500-550	407 nm	4.8 h*	59
<b>2</b>	635 nm	450 nm	3.5 h	71
<b>3</b>	530 nm / 635 nm	460 nm	2.4 d	71,72
<b>4</b>	423 nm / 635 nm	nd	25 min	73
<b>5</b>	>450 nm	410 nm	700 d	60
<b>6</b>	>500 nm	440 nm	nd	60
<b>7</b>	>500 nm	410 nm	nd	60

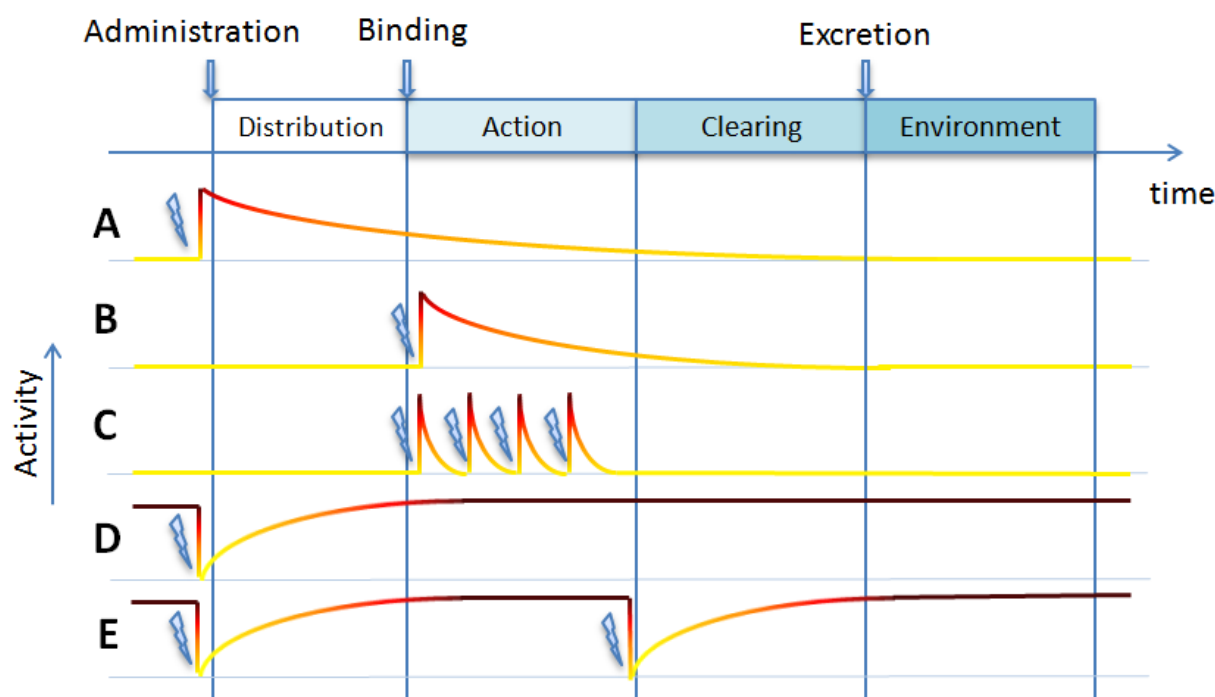
\* *cis* isomer is the thermodynamically stable one.

In particular, the tetra-*ortho*-substituted azobenzenes, introduced only recently, are notable, as it is possible to induce their *trans* to *cis* isomerization using light of longer wavelengths, e.g. by irradiating the red-shifted n- $\pi^*$  band of the *trans* isomer.<sup>71-73</sup> In a seminal study,<sup>71</sup> Woolley and co-workers have shown that a short, fluorescent, reporter peptide, cross-linked with compound **2** could be photoisomerized with red light ( $\lambda = 635$  nm), even in zebrafish embryos.

Based on these recent results, we envision the possibility of tuning the optical properties of photoswitches to match the “therapeutic spectral window” for photopharmaceuticals, building on important lessons learned from the field of clinically used photodynamic therapy.<sup>74</sup> Further developments in the use of two-photon absorption processes for molecular photoswitches that would directly enable the use of near-IR light in photopharmacology are equally important in this field.<sup>75,76</sup>

### 3.4. The half-life of thermodynamically unstable isomer

Tuning the rate of the thermal relaxation of the switched state constitutes one of the main advantages over using traditional approaches, as it opens the possibility of temporal control of drug activity, in addition to approaches based on the use of two different wavelengths of light (*vide supra*). Figure 8 presents possible therapeutic scenarios one can envision when exploiting thermal relaxation of a photoswitchable drug.



**Figure 8.** Possible therapeutic scenarios using photoswitchable bioactive compounds with a controlled half-life, that show their activity either in the thermodynamically unstable (A-C) or stable (D-E) state: A) Irradiation prior to administration; B) Irradiation at the point of action; C) Multiple irradiation cycles of a drug with a short half-life for the on-state; D) Irradiation prior to administration; E) Irradiation prior to administration and while the drug is being cleared.

The simplest scenario (Scenario A, Figure 8) involves the use of a photopharmaceutical agent that is biologically active in its thermally unstable state. Irradiation of the drug prior to its administration would result in its temporal activation. If the half-life of this state is chosen properly, the activity would be retained during the time the drug resides in the body of the

patient and would be lost by the time it is excreted. Since the irradiation occurs prior to administration, the lack of transparency of human tissue to the required wavelength is not a limitation. This scenario is especially attractive for preventing the build-up of active compounds in the environment (see Section 3.3). Alternatively, if the optical properties of the photopharmaceutical agent allow the activation within the body, scenario B (Figure 8) can be utilized to the same end. In cases where the drug exhibits severe cytotoxicity and precise control over its localization is required, installing a photoswitch with short half-life would allow scenario C (Figure 8) in which repeated activation (pulsing) in the desired area would result in spatially selective action.

The other scenarios (D and E, Figure 8) apply when the photopharmaceutical agent is active in its thermodynamically stable state. The adverse side effects could in this case be limited by irradiation-promoted, temporal inactivation prior to administration. If the timescale of the thermal relaxation is chosen correctly, the compound would regain most of its activity upon reaching the target (Scenario D, Figure 8). Additionally, irradiation could temporally inactivate the compounds before being cleared from the body to prevent toxicity in other tissues (Scenario E, Figure 8).

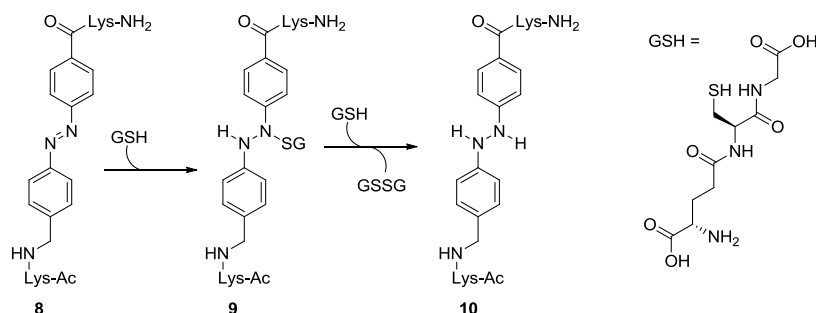
In recent years, major advances have been made in understanding the factors that determine the thermal stability of the photoswitched states, especially for azobenzenes. Previous approaches at constructing azobenzenes that can be switched with visible light focused on installing push-pull systems, which resulted in very fast thermal relaxation in polar solvents. While such systems are highly useful for approaches in which a fast drop in activity is desired (such as Scenario C, Figure 8), their general applicability is limited (Scenarios A, B, D and E, Figure 8).<sup>77,78</sup> Recently, the wavelength of absorption was decoupled from the stability of the thermodynamically unstable state. Molecular photoswitches were constructed in which the possibility of visible light *trans-cis* switching results from the red shift of the  $n-\pi^*$  band of the *trans* isomer, and its separation from the  $n-\pi^*$  transition of the *cis* isomer. This enables switching in both directions with visible light, while the thermal stability of *cis*-isomer is not compromised (Table 1). For example, compound *cis*-**4** relaxes thermally with a half-life in the range of minutes,<sup>73</sup> *cis*-**3** has a half-life of 53 h<sup>72</sup> and *cis*-**5** shows an unprecedented half-life of 700 d, i.e. it is thermally stable.<sup>60</sup>

The final factor that has to be considered when designing photopharmaceutical agents is that the kinetics of their thermal relaxation measured *in vitro* might not be the same as in the intercellular space and inside cells. This is caused mainly by reversible attack of cellular thiols (see section 3.5) on the *cis*-azobenzene, which leads to increased relaxation rates.<sup>79-81</sup> Furthermore, environmental effects in cells have been reported to affect this process.<sup>82</sup>

### 3.5. Stability in the cellular environment

The possible degradation of azobenzenes via enzyme<sup>83,84</sup>- or glutathione (GSH, Scheme 1)<sup>81</sup>-mediated reduction to the respective hydrazobenzenes is a source of ongoing concern. Especially the second possibility has been studied in more detail, since glutathione is present in cells in concentration of up to 10 mM.<sup>85,86</sup> Prompted by the observation that proteinogenic

thiols reduce azobenzenes,<sup>87,88</sup> the group of Moroder<sup>80</sup> conducted a thorough study on the stability of an azobenzene **8**, incorporated into a backbone of a short peptide, in the presence of varying concentrations of glutathione and over a wide pH range. They observed that reduction of azobenzene indeed proceeds at neutral and basic pH, and it is 100 times faster for the *cis*-isomer of **8**.



**Scheme 1.** Mechanism of the reduction of a model, azobenzene-bearing tripeptide, **8** by glutathione.<sup>81</sup>

The mechanism of the reduction, presented initially by Kosower,<sup>89</sup> is a two-step process, which involves a nucleophilic attack of the thiol(ate) on the diazo bond to form an adduct **9** (Scheme 1),<sup>81</sup> which reacts further with GSH to form the hydrazo compound **10** and GSSG,<sup>81</sup> an oxidized form of glutathione. Introduction of electron-donating substituents to the azobenzene could prevent the first step from occurring, by decreasing the electrophilicity of the diazo system. Indeed, the use of mildly electron-rich *p*-amido substituents by Woolley and co-workers, for example in compound **1**, resulted in systems that showed remarkable stability at high glutathione concentrations.<sup>59,82</sup> On the other hand, in the tetra-*ortho*-substituted azobenzenes<sup>71,72</sup> **2** and **3**, an opposite trend was observed: compound **2**, bearing four chloro substituents proved to be stable to reduction, while compound **3** with four strongly electron donating methoxy groups was slowly bleached by glutathione ( $t_{1/2}$  ~1h at 10 mM GSH).<sup>71</sup> This difference was rationalized by the higher basicity of the latter compound, which upon protonation undergoes the thiolate attack more rapidly.<sup>71</sup> By using methylthio groups instead of methoxy groups in compound **4** the reduction could be prevented.<sup>73</sup>

These examples show that, in general, azobenzene-based photopharmaceuticals are stable in reducing, cellular environment, at least on the timescale relevant for therapeutic applications, and that their stability can be tuned by variation in the substitution pattern. This is further supported by their use in *in vivo* experiments,<sup>71,82</sup> including the photoisomerization of azonium switches, based on compound **3**, in whole blood.<sup>90</sup> However, attention must be paid to the electronic properties of the substituents, as installing electron-withdrawing groups might lead to higher susceptibility to reduction within the cells. In any case, testing newly developed photoswitchable compounds for their stability in the presence of milimolar concentrations of glutathione is strongly advised.

### 3.6.Toxicity



The toxicity of azobenzenes, which have been used as dyes and manufactured on large scale, has been a subject of numerous studies and general guidelines for the design of non-toxic compounds have been established.<sup>91</sup> Briefly, the toxicity of azobenzenes may stem from three sources: (i) the cleavage to carcinogenic aromatic amines, which upon oxidation form species that bind to DNA covalently, (ii) metabolic oxidation of amine-bearing azobenzenes to toxic species, and (iii) oxidation to strongly electrophilic diazonium salts.<sup>91</sup> Therefore, in considering the toxicity of azo-based photopharmacological agents, not only the toxicity of the parent structure should be considered, but also the effects caused by the corresponding amines.

Despite these limitations, it should be mentioned that azobenzene-based compounds have been successfully applied as pro-drugs in therapies, as exemplified by sulfasalazine<sup>92</sup> and balsalazide,<sup>93</sup> food/drug/cosmetic additives, e.g. allura red AC<sup>94</sup> and diagnostic aids, such as anazoline.<sup>95</sup> These examples of successful applications of azobenzenes in medicinal contexts show that careful consideration of possible toxicity may allow the construction of biologically safe compounds.

### **3.7. Design principles: concluding remarks.**

A photoswitchable drug must fulfill a set of requirements in order to demonstrate its potency in enabling the use of light to control biological activity, : (i) the change in concentration of the more active isomer upon irradiation must be sufficient to provide a large difference in response; (ii) the isomerization should preferentially be achieved using deep-penetrating light of low cellular toxicity; (iii) the thermal re-isomerization to the thermodynamically stable form should proceed on the timescale relevant for the intended application; the photoswitchable drug should be (iv) water soluble in both photoisomeric states and (v) metabolically stable enough to reach its intended target; finally, (vi) neither the drug, nor its metabolites should show general toxicity.

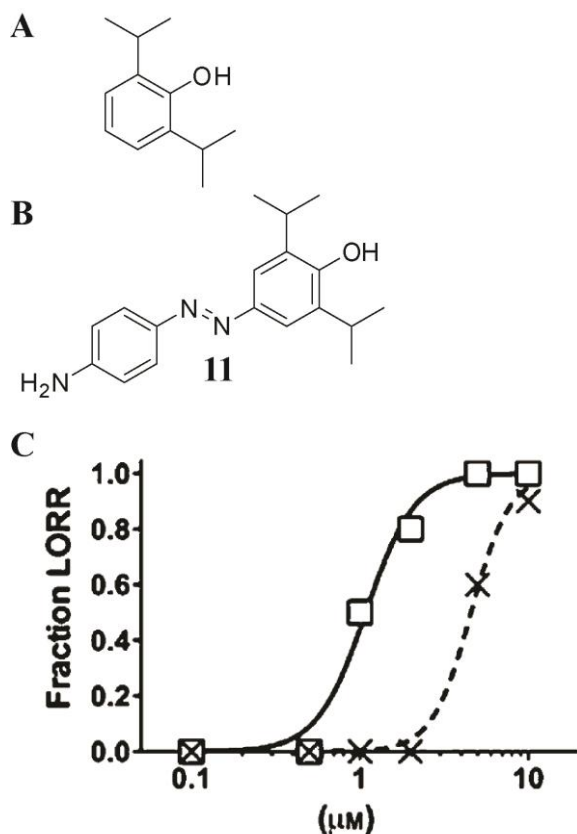
While in recent years major advances have been made in understanding factors that govern each of these requirements separately, it remains a challenge to fulfill all of them within one molecule. We envision that continued research efforts towards understanding the principles underlying the factors discussed above would result, in the years to come, in establishing primary architectures of photochromic elements that can be successfully incorporated into drugs to render them photoresponsive. There are also, of course, ample opportunities for employing other molecular photoswitches besides the azobenzenes, and we expect major developments in this regard. While the field of photopharmacology is still in its infancy, it is highly promising due to the versatility provided by molecular design.

## **4. Illustrative examples**

The second part of this perspective describes selected examples of photopharmaceuticals, with the focus on the design principles used (see section 3.1) and the attempts at making the compounds compatible with the requirements for successful therapeutic use (see Sections 3.2-3.6). For a more comprehensive overview on the photocontrol of biological systems by the application of molecular switches, the reader is referred to other reviews.<sup>21,96</sup>

Pioneering work in the field of photopharmacology has been conducted by the group of Erlanger and Nachmanson, who studied azobenzene-based inhibitors of acetylcholinesterase in the 1960s.<sup>97,98</sup> In the following decades, important contributions included photoswitchable inhibitors of proteases<sup>99-101</sup> and mitochondrial complex I.<sup>102</sup>

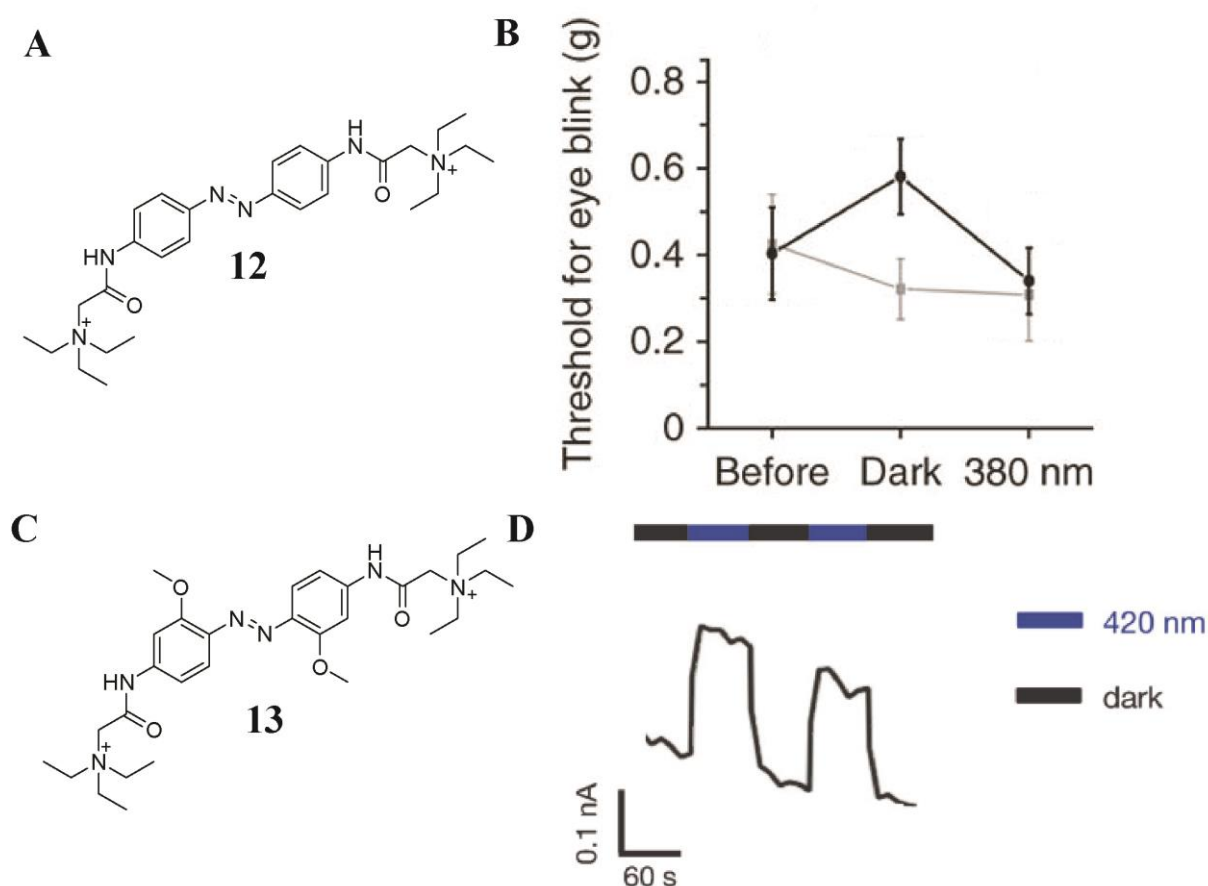
Recently, several successful examples<sup>49,77,103-107</sup> were reported by Trauner and co-workers, including the development of a photoswitchable propofol analog.<sup>49</sup> Propofol (Figure 9A) is widely used as an anesthetic and it derives its effect by potentiating GABA-induced currents.<sup>49</sup> The first strategy outlined above was taken in the design of the photoswitchable compound i.e. coupling of a photoswitch to the pharmacophore of propofol (Figure 4A). Compound **11** (Figure 9B) was obtained by introducing a 4-aminophenyldiazo group at the *para* position of the aromatic moiety of propofol. The amino substituent in the *para* position caused a red-shift in the absorption spectra of the azobenzene and **11** could be switched from the *trans* to the *cis* isomer by irradiation with 360-450 nm light. The electron-donating substituent on the azobenzene resulted in a significant decrease in the thermal-stability of the *cis*-isomer, and as a consequence a fast, thermal *cis-trans* isomerization occurred once the 360-450 nm light source was removed. The anesthetic effect of both photoisomers of compound **11** was evaluated using *Xenopus laevis* tadpoles. In this experiment, the animals were studied for loss of righting reflex (LORR).<sup>108</sup> This reflex corrects the orientation of the body when it is taken out of its normal position and the loss of this reflex is a standard assay for anesthesia. The tadpoles placed in aqueous media containing compound **11** showed an LORR with an EC<sub>50</sub> of 1.1  $\mu$ M, which was comparable to the EC<sub>50</sub> of propofol itself. When the tadpoles were irradiated with light (360-370 nm), the EC<sub>50</sub> increased by more than 4-fold to 4.6  $\mu$ M (Figure 9C). In a second experiment, tadpoles were placed in medium containing 3  $\mu$ M of compound **11**, which resulted in LORR. Subsequently, the medium was illuminated with UV-light and all animals spontaneously righted themselves. This experiment shows that even though both photoisomers have similar biological activity, an on/off event can be realized by carefully control of the concentration (Figure 5D). Additionally, it proves that an existing drug can be converted into a photoswitchable analog and drug activity can be regulated *in vivo*.



**Figure 9.** A photoswitchable anesthetic. A) Structure of propofol; B) Structure of compound **11**; C) Dose-response curve of compound **11** in its *trans*-form (squares) and in its *cis*-form (crosses). Figure adapted with permission from ref. 49. Copyright 2012 Wiley-VCH Verlag GmbH & Co. KGaA.

In another recent example by the groups of Trauner and Kramer, a photoswitchable nociception regulator was described.<sup>105</sup> Nociception is involved in the processing of noxious stimuli by nociceptors.<sup>109</sup> These receptors are expressed in neurons and contain ion channels that can be blocked by quaternary ammonium groups. The first design strategy was again taken in developing a photoswitchable nociception regulator (Figure 4A), where an azobenzene moiety was incorporated into a lidocaine analog, to yield compound **12** (Figure 10A). The effect of compound **12** on pain sensation *in vivo* was evaluated using a pain-avoidance experiment with rats. The von Frey hair test was conducted, which assays the blinking response after mechanical stimulation of the cornea.<sup>110</sup> Compound **12** was applied topically in combination with capsaicin to one eye and capsaicin alone to the other. Then, increasing force was applied to the eye. In the dark, a force of almost 0.6 g was necessary to make the eye blink in the presence of 20 mM of compound **12**, while the control eye without compound **12** already blinked when a force of 0.3 g was applied, indicating that compound **12** could alter pain sensation *in vivo*. When the eye was irradiated at 380 nm, which switches compound **12** from the *trans* to the *cis* isomer, the eye with compound **12** already blinked at a force of 0.3 g, as with the control eye without compound **12** (Figure 10B). In a follow-up report<sup>106</sup> the structure of compound **13** was modified to allow it to undergo photoisomerization using visible light, instead of UV light, i.e. an *ortho,ortho'*-dimethoxy analog of **13** (Figure

10C). This compound could be photoisomerized from its *trans* to its *cis* form with visible light at 420 nm. To test its activity, Shaker-IR K<sup>+</sup> currents were recorded in the presence of 350  $\mu$ M compound **13** in the dark or when irradiated at 420 nm on HEK293 cells using voltage-clamp recordings (Figure 10D). A significant increase in current was observed when the cells were irradiated at 420 nm light as compared to the dark. These studies show how a previously designed photoswitchable drug can be further developed to optimize its photochromic properties while still maintaining its biological activity.

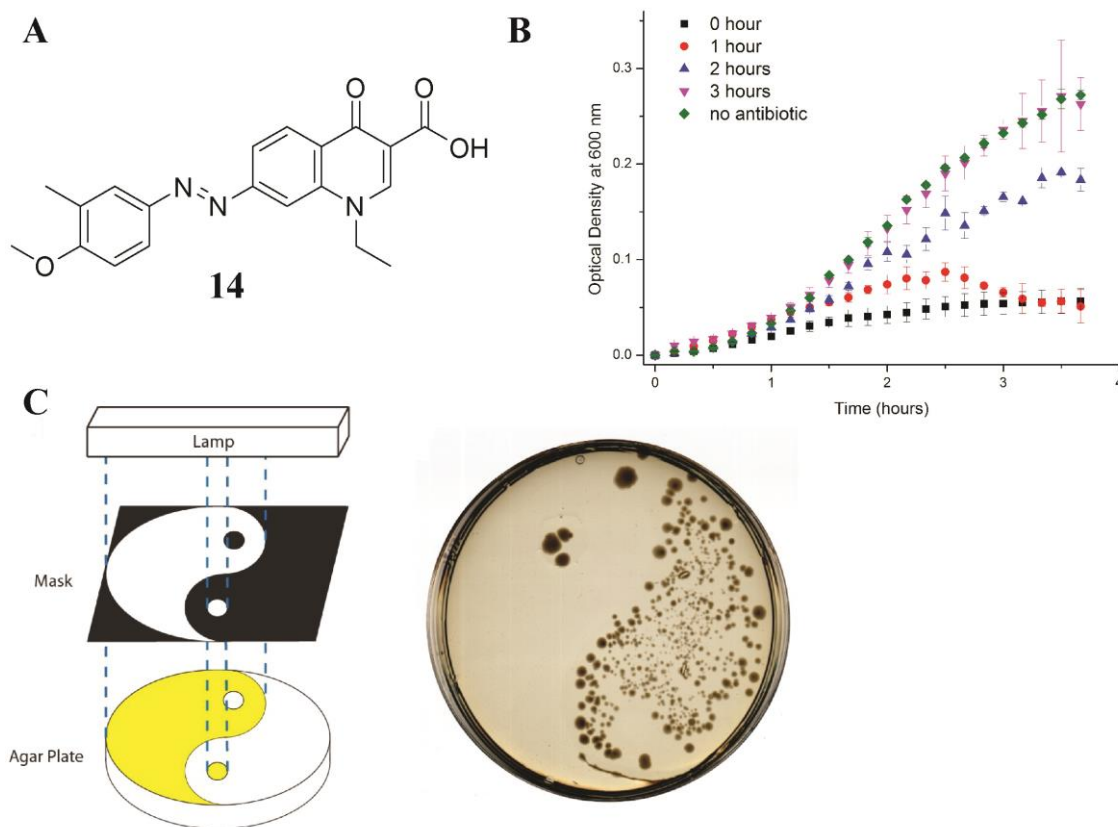


**Figure 10.** Photoswitchable nociception stimulation by azobenzenes **12** and **13**. Molecular structures of compound **12** and **13** and their biological activity. A) Molecular structure of compound **12**. B) The threshold of eye blinking in the dark and when exposed to 380 nm light when capsaicin (grey line) or capsaicin and compound **12** (black line) were applied topically to the eye of a rat. C) Molecular structure of compound **13** D) Voltage clamp recordings of Shaker-IR K<sup>+</sup> channels in HEK293 cells incubated with compound **13** in the dark and irradiated at 420 nm. Figures adapted with permissions from refs. 105 and 106. Copyright 2012 Nature Publishing Group (ref. 105); Copyright 2012 Wiley-VCH Verlag GmbH & Co. KGaA (ref. 106).

Recently, Feringa and co-workers reported a photoswitchable antibiotic.<sup>50</sup> SARs of the derivatives of the well-known (fluoro-)quinolones were used to design a series of compounds, which were prepared using the first design strategy also (Figure 4A). Quinolones are broad-spectrum antibiotics that are frequently used in pharmacotherapy.<sup>111</sup> An azobenzene

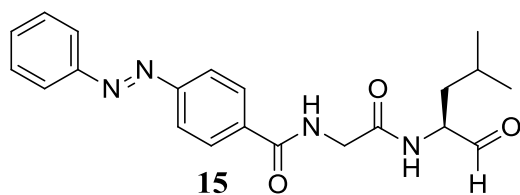
photoswitch was incorporated into the quinolone pharmacophore and various substituents were introduced on the aromatic ring of the azobenzene. The minimum inhibitory concentration (MIC) was determined for all compounds in the dark and after irradiation at 365 nm, which switches the compound from the *trans* to the *cis* isomer. Compound **14** (Figure 11A) had a MIC value of 16 µg/mL before irradiation and 2 µg/mL after irradiation when tested on the Gram-positive bacterium *M. luteus*. Additionally, compound **14** showed to be more active in the irradiated form against *E. coli*, indicating that this photoswitchable quinolone retained its broad-spectrum character. The thermodynamically unstable *cis*-form was the most active isomer. Irradiating the stable *trans*-isomer at 365 nm resulted in temporary activation only, as its activity decreased in the absence of light due to thermal *cis-trans* isomerization (Figure 8, scenario A) with a half-life of ca. 2 hours. The authors showed that the *cis-trans* isomerization lead to a loss in activity by determining the antibacterial activity over time after irradiation (Figure 11B). This temporary activation means that, in the case of use *in vivo*, the activity is likely to be lost before the drug is excreted from the body, preventing the build-up of an active antibacterial substance in the environment and avoiding the emergence of resistance. By variation of the substituents on the aromatic ring, it might be possible to tune the half-life for optimal activity in the body and minimal activity outside the body.

Furthermore, it was shown that antibacterial activity could be locally increased by irradiating a section of an agar-plate containing compound **14**.<sup>50</sup> After irradiation, *E. coli* was streaked on the plate and incubated overnight. Bacterial growth was only observed at the section that was not exposed to light (Figure 11C). This shows how precisely a drug can be activated in time and space using this method. When photoswitches are used that can be addressed with longer wavelengths, activation of drugs can, in principle, be achieved inside the body. This example also shows how the half-life of *cis-trans* isomerization can be exploited to lose drug-activity over time (see Section 3.4).



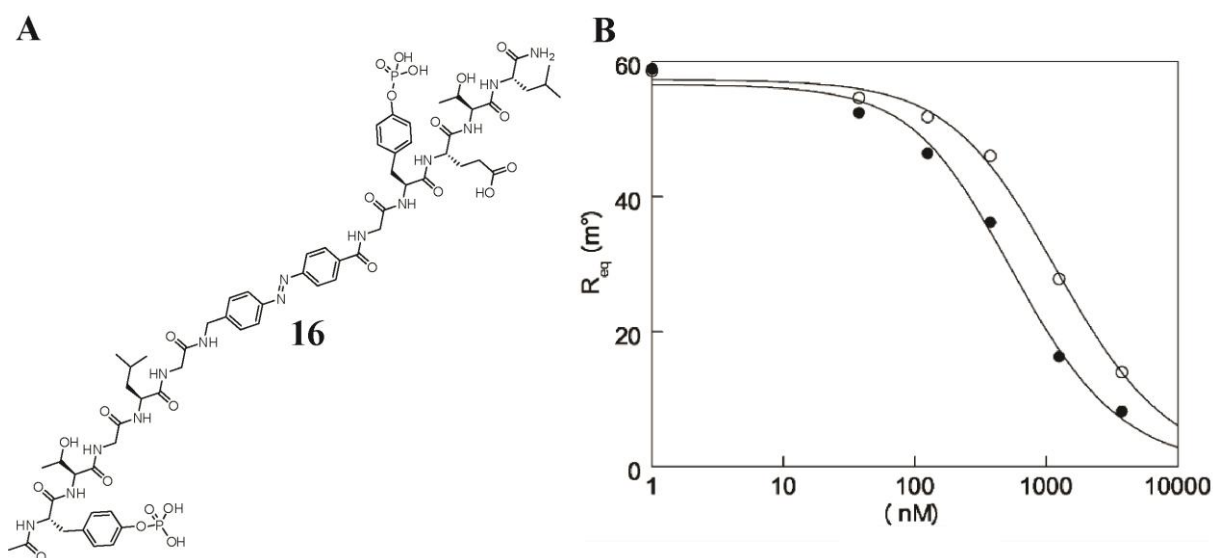
**Figure 11.** Photocontrol of antibiotic activity. A) Molecular structure of compound **14**; B) Bacterial growth curves of *E. coli* incubated with 40  $\mu$ M of compound **14** at various times after irradiation. After 3 hours, antibacterial activity was lost completely due to thermal *cis-trans* isomerization; C) Irradiation of compound **14** in an agar plate resulting in local activation of antibiotic activity, preventing bacterial growth locally.<sup>50</sup>

In 2007 Abell and co-workers described photoswitchable anticataract agents.<sup>112</sup> A cataract is a condition that is characterized by clouding of the lens in the eye which impairs vision. This disease can be caused by over-activation of a protease, called calpain, which catalyzes the breakdown of lens proteins.<sup>112</sup> Photoswitchable calpain inhibitors were designed based on modeling of the active site of calpain. The inhibitory activity of compound **15** (Figure 12) was tested on calpain using a standard, fluorescence-based assay. The non-irradiated compound **15** had an  $IC_{50}$  value of 45 nM, while after irradiation at 340 nm the  $IC_{50}$  value was 175 nM. Such a design could be used to first deactivate the inhibitor by irradiation with UV-light, avoiding side-effects and subsequently activating the drug when it has reached the target. The elegance of this approach stems from the fact, that the target is the lens in the eye and the drug could be naturally activated by illumination with visible light, minimizing toxicity-related problems (see section 3.3).



**Figure 12.** Molecular structure of compound **15**

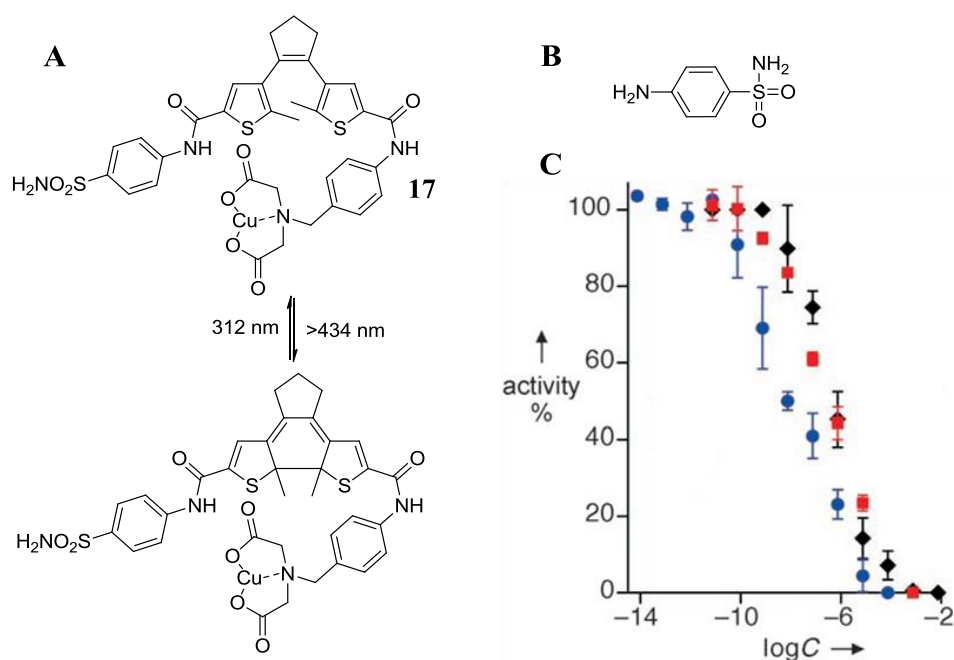
The group of de Mol and Liskamp have reported photoswitchable ITAM peptidomimetics.<sup>56,113</sup> Spleen tyrosine kinase (Syk) plays an important role in immunological receptor signaling and therefore is involved in allergic reactions and immune responses.<sup>114</sup> Syk is activated by binding to a so-called ITAM motif on the IgE receptor, which is possible only when the motif is diphosphorylated. Over-activation of Syk induces allergic responses, which renders Syk an interesting target for anti-allergic therapy. To develop a photoswitchable ITAM peptidomimetic, the second design strategy was used, where two pharmacophores were linked by a photoswitch moiety (Figure 4B), in this case an azobenzene. The ITAM motif consists of a small peptide comprising of amino acids that are essential for Syk binding, which were considered as the pharmacophores, and of amino acids that are not essential for binding, which were considered as the spacer. Taking this in consideration, compound **16** (Figure 13A) was designed, featuring an azobenzene at the center of the molecule, flanked by binding peptides.<sup>56</sup> SPR competition experiments were performed to test if there was a difference in binding affinity between the *trans* and *cis* isomer (Figure 13B). A dose-response curve was obtained for compound **16** before and after irradiation at 366 nm, showing a small, yet significant, change in binding affinity upon irradiation. However, irradiation at 366 nm resulted in a maximum of only 60% *cis* isomer. If this percentage could be increased, the difference in binding affinity would be larger. Nevertheless, this example shows how the second design strategy (Figure 4B) can be successfully exploited to provide a photoswitchable pharmaceutical.



**Figure 13.** A photoswitchable ITAM peptidomimetic A) Molecular structure of compound **16** B) SPR competition experiment with Syk (50 nM) with compound **16** when irradiated at 366

nm (white circles) and non-irradiated (black circles). Figure adapted with permission from ref. 56. Copyright 2008 Elsevier B.V.

This design, in which a molecular photoswitch is used in a drug as a linker that binds two moieties that interact with the target (Figure 4B), is not limited to multivalent targets. Carbonic anhydrases are a family of zinc-containing enzymes, which have been used as targets in many pharmacological therapies of conditions such as hypertension, convulsion, epilepsy and diabetes, among others.<sup>115</sup> The groups of König and Branda have described<sup>116</sup> compound **17** (Figure 14A), a photoswitchable inhibitor of human Carbonic Anhydrase I (hCAI), whose design is inspired by the observation<sup>115</sup> that the inhibitory activity of sulfanilamide (Figure 14B) is enhanced by covalent attachment of a copper complex. The sulfanilamide residue interacts with the active site, while copper is believed to coordinate to the imidazole side chains of the histidine residues that are present on the surface of hCAI. Since the distance between the  $\text{Zn}^{\text{II}}$  ion in the active site and the histidines is fixed, it was assumed that installing a diarylethene linker between the sulfonamide and the Cu complex in compound **17** might allow for the photocontrol of drug activity.



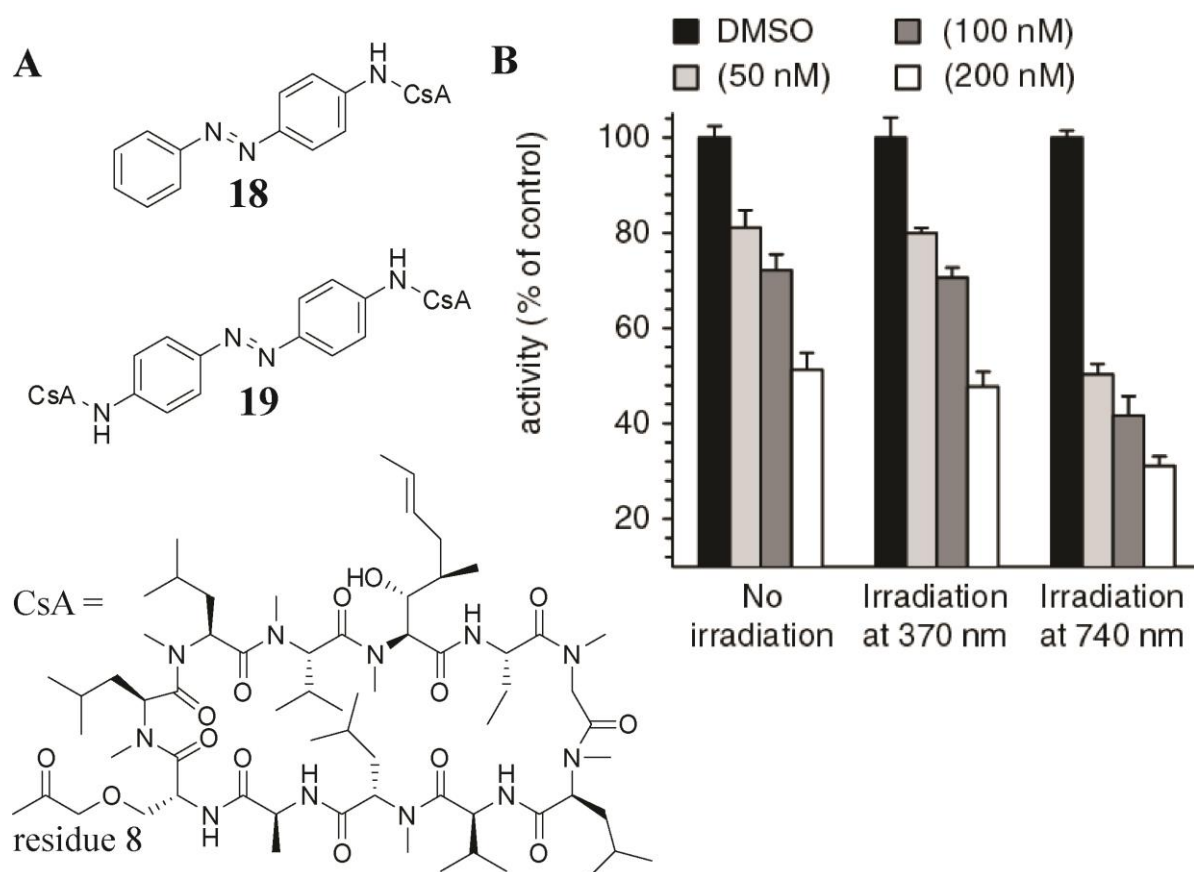
**Figure 14.** Photocontrolled inhibition of hCAI; A) Photoisomerization of compound **17**. B) Sulfanilamide, a weak inhibitor of hCAI; C) Activity profile; ● – open **17**; ■ – closed **17**; ◆ - Sulfanilamide. Reprinted with permission from ref. 116. Copyright 2008 Wiley-VCH Verlag GmbH & Co. KGaA.

Irradiation of the open form of **17** at 312 nm (Figure 14A) resulted in complete (>99%) photocyclization to the closed isomer, which could be reversed fully by irradiation with visible light. Remarkably, as shown in Figure 14C, closed-**17** showed very high hCAI inhibitory activity ( $\text{IC}_{50} = 8 \text{ nM}$ ) when compared with the parent compound, sulfanilamide ( $\text{IC}_{50} = 460 \text{ nM}$ ), and the photocyclization resulted in a significant loss of activity (closed-**17**



shows IC<sub>50</sub> value of 400 nM). This effect was attributed to the large difference in conformational flexibility between the closed and open forms of **17** and the bidirectional switching (see Section 3.2). This example highlights the strength of the second approach to designing photopharmaceuticals (Figure 2B). It also shows the as of yet unexplored potential of diarylethenes as photoregulating agents used in photoswitchable drugs.

Both major design strategies (Figure 4A and B) were employed by Fischer and coworkers<sup>51</sup> to obtain a photoswitchable immunosuppressant, using a cyclic non-ribosomal peptide, cyclosporine A, as a template. This immunosuppressive drug binds to the protein cyclophilin and the resulting cyclosporin A-cyclophilin complex inhibits calcineurin. Under normal conditions, calcineurin is responsible for immuno-activation<sup>117</sup> and its inhibition results in immunosuppression. The initial design (compound **18**, Figure 15A) was based on the first strategy (Figure 4A); an azobenzene photoswitch was conjugated with cyclosporin A. Based on SAR studies, it was known that modification at residue 8 (Figure 15A) of the cyclic peptide of cyclosporine A would not result in total loss of activity. Compound **18** could be switched by irradiation at either 370 nm or 740 nm, using two photon photoisomerization, resulting in almost 95% *cis*-isomer. When testing the inhibitory potencies of compound **18**, only a moderate difference in activity was observed between the two photoisomers.<sup>51</sup> To further increase this difference, another compound was synthesized using the second design strategy (Figure 4B). Two cyclosporine A molecules were linked together by an azobenzene moiety resulting in compound **19** (Figure 15A). To test its activity, inhibition of endogenous cyclophilin in blood lysate was measured (Figure 15B). The *trans*-isomer was able to inhibit cyclophilin activity up to 50% at 200 nM concentration. When the samples were irradiated at 370 nm, the same results were obtained as without exposure to light (Figure 15B). However, when the samples were irradiated at 740 nm, an increase to 70% inhibition was found (Figure 15B). This increase can be attributed to a higher activity of the *cis*-isomer. Because the experiments were conducted in blood lysate, light at 370 nm is unable to penetrate the sample and photoisomerize compound **19** (see Section 3.3). At 740 nm, the penetration depth is greater and for this reason, *trans-cis* isomerization can occur and higher inhibitory activity is observed. This remarkable example shows how longer wavelength light can be used to switch between states of different activities in a biological sample and demonstrates the potential for medical applications.



**Figure 15.** A photocontrolled immunosuppressant. A) Molecular structures of compound **18**, compound **19** and cyclosporine A; B) Cyclophilin activity after incubation with different concentrations of compound **19** in blood lysate irradiated with 370 nm light, 740 nm light or no irradiation. Figure adapted with permission from ref. 51. Copyright 2009 Nature Publishing Group.

## 5. Outlook

Regulating biological processes with light, by employing photochromic molecules, offers fascinating possibilities, with far-reaching implications in chemical biology, pharmaceutical research and medicine. Although the idea of using photoswitchable enzyme inhibitors has been demonstrated as early as 1969,<sup>97</sup> it is only recently that this approach is emerging to the stage where it shows its potential in future therapeutic applications. The development of novel or improved photochromic compounds, in the last years, is particularly promising and provides important tools for photopharmacology, as highlighted by the development of visible (less harmful) light-switchable azobenzenes with long lifetimes of the *cis*-isomer, by precise positioning of substituent on the photochromic unit.<sup>59,60,71-73</sup> It is accompanied, in parallel, with the introduction of novel approaches towards application of photopharmaceuticals in medical treatment of e.g. bacterial infections, without inducing microbial resistance.<sup>50</sup> Alternatively,

the interference with neural processes such as nociception regulation,<sup>105</sup> offers fascinating prospects for control of more complex behavioral functions.

Major developments in the design of light-responsive molecular tools are needed to fulfill all the prerequisites for pharmacotherapy, but we have only started to explore the vast structural space of molecular photoswitches (see Section 3.7). In particular, molecular photoswitches are needed that could be photoisomerized in both directions with light in the therapeutic window (600-1200 nm) with good control over the thermal stability of the less stable isomer. The use of two-photon excitation processes provides a viable alternative. Other requirements in molecular design include water solubility, low toxicity and metabolic stability, without compromising the selectivity for the biological target. The control of the thermal stability of the isomers of the bistable chromophore will also be a key design issue. Interference with for instance, neural processes might require short-living *cis*-isomers and pulsed sequences upon irradiation, allowing for fast back-isomerization. Developments in these areas are eagerly awaited and will set the milestones on the way to applicable photocontrolled drugs.

The search for novel and unconventional approaches to dynamically controlling the activity of drugs in time and space has just started. With a plethora of optical molecular switches and pharmacophores available and a multitude of drug targets, the possibilities for smart and responsive pharmaceuticals are numerous. Merging the fields of photoswitches and potential drugs requires an exquisite molecular design, but offers a great playground to control biological function in a non-invasive way, including receptors, enzymes, replication and transcription, metabolic pathways, cellular networks, transport and sensory and neuronal processes. An obvious prerequisite for the success rate of these approaches is the design of the photopharmaceuticals which would differ considerably in their biological activity between the two photoisomeric states.

Implementing photopharmacology in the pharmaceutical industry to attain photoswitchable drugs that could be used in clinical practice will be presented with limitations and challenges, certainly those common in drug development. An important additional one is that optimization of the molecular structure of the drug to obtain high activity has a limitation, because a photoswitchable moiety needs to be present in the structure. However, photopharmacology might be an attractive option for certain cases where drug activity is only locally desired, because of severe side effects, like in the case of cancer therapy. Furthermore, introduction of a photoswitchable moiety into the structure of a known drug does not necessarily lead to activity loss as was shown in certain examples discussed above.

We envision that, in the years to follow, numerous new applications for photopharmaceuticals will emerge. With optimized architectures of photoswitches in hand and the advantage of high spatiotemporal control, it will provide both the incentive and the means to bring photopharmacology to a bright future, beyond the current state of “proof-of-principle” and in the realm of novel therapeutic applications in the clinic.

## ACKNOWLEDGEMENTS:

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## TOC ENTRY:

